ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE MICROBIOLOGY

# AN IMPROVED METHOD FOR EXTRACTION OF HETEROLOGOUS DNA FROM ENVIRONMENTAL SAMPLES FOR THE CONSTRUCTION OF METAGENOMIC LIBRARIES

*Corresponding Author*

**DR. PINDI PAVAN KUMAR**

**Associate Professor and Head, Department of Microbiology, Palamuru University, Mahabubnagar – 509001 (A.P.) India.**

## ABSTRACT

Soil represents a diverse group of microorganism, which has frequently been used to isolate and explore and exploit microbes for industrial, environmental and agriculture applications. Presently, several methods are being used to isolate the DNA from environmental samples. This paper deals with an improved method developed for the isolation of heterologous DNA from environmental soils or sediments. This approach consists of the direct extraction of large fragmented nucleic acids from soil followed by purification. Cell lysis is a critical step in soil metagenomic DNA extraction. Extraction procedure was optimized with series of steps, which involved gentle mechanical lysis and number of freeze-thawing cycles in liquid nitrogen and with variations in incubation period and temperature. A comparison of the optimized protocol with other existing protocols and with commercially available kit suggests that protocol described in this report would be more efficient and yields high quantity and quality DNA from environmental samples.

## KEYWORDS

Genomic DNA, Soils, Metagenomic libraries, Molecular methods

## INTRODUCTION

Genomic DNA isolation from environments has become increasingly popular as the phylogenic or the population genetics or metagenomics research become increasingly important owing to environment concerns. For these molecular genetic studies, it is important to isolate large fragment genomic DNA. Many molecular biology companies are producing specific genomic DNA isolation kits for a range of biological materials. Most of these commercial products use columns and DNA-binding resin, which breaks the genomic DNA into small pieces.

Microorganisms are the most ubiquitous organisms on earth and represent in all habitats including terrestrial sub surface, marine and even from stratosphere. They play a vital role in the bio-prospecting of enormous number novel bioactive molecules. The vast majority of enzymes and antimicrobial products have been isolated from microorganisms by classis approach. Only 1% of microorganisms were isolated and characterized so far and 99% remain unexplored. Construction of complex metagenomic libraries will be a big asset for identification of industrially important novel enzymes. Isolation of high molecular weight genomic DNA from environment has facilitated the cloning of DNA into BAC vector [1]. Cloning of large regions of the genomes of as yet uncultured bacteria will provide a route to study the genomes of uncultured bacteria.

Many different methods are available for the isolation of genomic DNA. In general conventional genomic DNA isolation methods involve disruption and lysis of the cell wall or membrane followed by the removal of proteins and other contaminants and finally recovery of the large fragmented DNA. The choice of the method depends on many factors like purity, time and expenses.

In the current study was undertaken to have an efficient protocol for the isolation of high purity and unshared genomic DNA from environmental samples to construct high quality of metagenomic libraries.

## MATERIALS AND METHODS

### *DNA isolation from soil*

Composite soil samples were collected from the Palamuru University campus promises, Mahabubnagar district, Andhra Pradesh, India. Heterologous DNA was isolated from three soil samples by employing the improved method. Sieved fine soil of 3 grams was used for extraction with 6ml of extraction buffer (100mM Tris-Cl, pH 8.0, 100mM Na-EDTA, pH 8.0, 1.5M NaCl). After gentle mixing, 0.5mg of proteinase K was added. All the tubes (Folocn tubes, BD Biosciences) were incubated horizontally at 37° C with shaking at 180 rpm for 30 min. After incubation, 3 ml of 20% SDS (Sodium Lauryl Sulphite) was added and incubated at 65°C for 90 min. The efficiency of lysis was determined by direct cell counts with phase contrast microscope and by acridine orange direct cell counts with fluorescence microscopy. Three cycles of freezing in liquid nitrogen and thawing at 65°C in water bath were conducted to release the DNA from the microbial cells present in the soils or sediments. The samples were centrifuged at 6000 rpm for 10 min at room temperature. The supernatant was transferred into fresh Folcon

This article can be downloaded from www.ijpbs.net

tubes. The remaining soil pellet was treated three times with extraction buffer and 20% SDS for re-extraction. For the above collected 3 ml of supernatant, half of the volume of 30% PEG (Poly Ethylene Glycol) and 1.6 M NaCl in 1:1 ratio was added and mixed. Then sample was incubated at room temperature for overnight. After the incubation, the sample was centrifuged at 10,000 rpm for 20 min at room temperature. Three ml sample of top aqueous layer was collected and mixed with equal volume of Tris saturated phenol, Chloroform and iso amyl alcohol at the ratio of 25:24:1 and centrifuged at 5000 rpm for 5 minutes. A 2.5 ml portion of the resulting extract was further extracted with an equal volume of chloroform, iso amyl alcohol mix (24:1) and centrifuged at 5000 rpm for 5 minutes. Finally, nucleic acids in the extracted aqueous phase were collected carefully without disturbing the middle layer. To avoid the shearing of DNA cut tips were used. Collected supernatant was treated with 0.6 volume of cold isopropanol and incubated at room temperature for 2 hours. After the incubation period the sample was centrifuged at 14,000 rpm for 15 minutes at room temperature. Supernatant was discarded and kept it for vacuum dry. Finally, DNA pellet was dissolved in autoclaved Tris Hcl buffer (10 mM; pH8.5) or in TE buffer (20mM Tris-HCl, 1mM EDTA, pH 8.0). The RNA molecules in the crude extracts were removed by incubating with heat-treated pancreatic RNase A (final concentration 0.2 μg/μl) for 2 hours at 37°C. The RNA free DNA was then purified by ethanol precipitation. Supernatant was removed, air dried and finally, pellet was dissolved in TE buffer. Concentration of DNA was estimated by agarose gel electrophoresis and ultraviolet spectrophotometer. The final purified DNA was compared with reference bands containing 25-400 ng lambda phage DNA. Spectrophotometric $A_{260}/A_{280}$ rations of the final soil DNA were 1.97 and 1.80.

***Metagenomic library construction***

The environmental DNA isolated through different methods has been used for the isolation methods have been reported for the construction of metagenomic libraries [2, 3]. In the present study, the purified DNA from the soil of Palamuru University campus promises was further used for construction metagenomic libraries. The isolated DNA was partially digested by BamHI (Fig. 2) and the resulting fragments of 3 Kbp to 18 Kbp size were ligated with the BAC vector and transformed in to EPI300 *E. coli* strain (Epicentre) and grown on LB medium 25 ug ml-1 of chloromophenicol overnight. The resulting library was stored in 15% glycerol in 396 well storage plate at -80°C.

## RESULTS AND DISCUSSION

Cell lysis is a crucial step in soil heterologous DNA extraction. Presently, several methods are available for extraction of environmental DNA. Chemical or enzymatic or mechanical disruption methods still remain elusive and extraction bias still exists [4]. Bead-beating-SDS method [5] allowed the high yield DNA, but resulted in increased DNA shearing when compared to the gentle freezing-thawing lysozyme method described by Tsai and Olson. Variations also exist in the number of freeze-thaw cycles and the incubation time and temperature [6-8].

Cell lysis generally combines treatments with detergent and lytic enzymes. The most widely used detergent is sodium dodecyl sulphate (SDS). [5, 9-12]. Lysozyme is the most commonly used enzyme for mucopeptide hydrolysis a polysaccharide component of the bacterial cell wall that gives rigidity and osmotic protection to the cell. Some proteases, proteinase K [13, 14] will help to free nucleic acids.

Chemical lysis, enzymatic action and freeze-thawing attributed to increased cell lysis. The number of freeze-thaw cycles and the incubation time in liquid nitrogen and in water bath at different temperature can vary [6-9, 13, 15-18]. Sodium Dodecyl Sulphate (SDS) has been the most widely used detergent for breaking down the phospholipid bilayer of the cell membrane [19]. Percentage of SDS and incubation time and temperature also effects on the cell lysis. Gray and Herwig [20] recommended combined chemical and mechanical lysis but our investigations, showed an efficient cell lysis with the combination of chemical, enzymatic and freeze-thawing. By using the three thermal shocks at -196° C and 65° C with lysozyme and SDS obtained 92% of cell lysed as measured by direct Acridin orange cell counts.

Organic extraction is a conventional technique that uses organic solvents to extract contaminants from cell lysates [5, 18]. The correct salt concentration and pH must be used during extraction to ensure that contaminants are separated into the organic phase and that DNA remains in the aqueous phase. It is found that pH 8.0 of the DNA extraction buffer more efficient than earlier reports [4]. Cut tips also play a vital role to avoid the shearing of DNA, while collecting the DNA from aqueous layer. Precipitation of DNA is also a crucial step to discard the contaminants to get high purity of DNA by using the sodium acetate and alcohol.

In comparative study between several extraction methods [21], our modifications allowed to get high quality of DNA yield without shearing when compared to Tsai method [18] and high yield and good quality of DNA when compared to Jacobsen CS method [22] (Fig. 1). Our modified method which includes gentle freeze-thawing, optimized incubation time and temperature and use of the cut-tips have significantly reduced the DNA shearing and also increased the DNA yield (Fig. 1).

The method is very simple for isolation of environmental DNA from different habitats without contamination of humic acid and other substances. Further, the extracted DNA was of high molecular weight and sufficiently pure for restriction enzyme digestion (Fig. 2) and construction of metagenomic libraries.

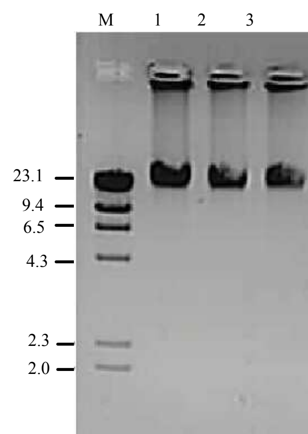


**Figure 1**

***Genomic DNA isolated from the soil of Palamuru University campus promises, Mahabubnagar district, Andhra Pradesh. India. M, HIndIII digest of Lambda DNA ladder; 1, DNA isolated from our modified method; 2, DNA isolated using protocol described by Tsai et. al. [18]; 3, DNA isolated using protocol described by Jacobsen CS et. al. [22].***

**This article can be downloaded from www.ijpbs.net**

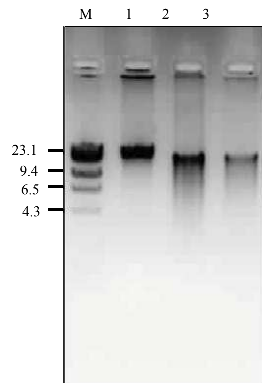


**Figure 2**

***Restriction digestion of isolated DNA. M, HIndIII digest of Lambda DNA ladder; 1, undigested genomic DNA isolated from modified method; 2, partially digested DNA by BamHI; 3, extensively digested DNA by BamHI.***

## CONCLUSION

In conclusion, we report an efficient modified protocol for isolation of high amount, un-sheared and good quality of genomic DNA for the construction of metagenomic libraries.

## ACKNOWLEDGEMENTS

Author is grateful to Prof. V. Gopal Reddy, Vice-chancellor and Prof. K. Venkata Chalam, Registrar, Palamuru University, Mahabubnagar (A.P) for their support and encouragement.

## REFERENCES

1. Berry AE, Chiocchini C, Selby T, Sosio M, Wellington EM (2003) Isolation of high molecular weight DNA from soil for cloning into BAC vectors. FEMS Microbiol Lett 223: 15-20
2. Harnpicharnchai P, Thongaram T, Sriprang R, Champreda V, Tanapongpipat S, Eurwilaichitr L (2007) An efficient purification and fractionation of genomic DNA from soil by modified troughing method. Lett Appl Microbiol 45: 387-391
3. Sharma PK, Capalash N, Kaur J (2007) An improved method for single step purification of metagenomic DNA. Mol Biotechnol 36: 61-63
4. Frostegard A, Courtois S, Ramisse V, Clerc S, Bernillon D, Le Gall F, Jeannin P, Nesme X, Simonet P (1999) Quantification of bias related to the extraction of DNA directly from soils. Appl Environ Microbiol 65: 5409-5420
5. Ogram A, Sayler GS, Barkay T (1987) The extraction and purification of microbial DNA from sediments. Journal of Microbiological Methods. Journal of Microbiological Methods 7: 56 - 66
6. Borneman J, Skroch PW, O'Sullivan KM, Palus JA, Rumjanek NG, Jansen JL, Nienhuis J, Triplett EW (1996) Molecular microbial diversity of an agricultural soil in

Wisconsin. Appl Environ Microbiol 62: 1935-1943
7. Clegg CD, Ritz K, Griffiths BS (1997) Direct extraction of microbial community DNA from humified upland soils. Lett Appl Microbiol 25: 30-33
8. Porteous LA, Seidler RJ, Watrud LS (1997) An improved method for purifying DNA from soils for polymerase chain reaction amplification and molecular ecology applications. Mol Ecol 6: 787-791
9. Bruce KD, Hiorns WD, Hobman JL, Osborn AM, Strike P, Ritchie DA (1992) Amplification of DNA from native populations of soil bacteria by using the polymerase chain reaction. Appl Environ Microbiol 58: 3413-3416
10. Kuske CR, Banton KL, Adorada DL, Stark PC, Hill KK, Jackson PJ (1988) Small-scale DNA sample preparation method for field PCR detection of microbial cells and spores in soil. . Appl Environ Microbiol 64: 2463-2472
11. Selenska S, Klingmuller W (1991) DNA recovery and direct detection of Tn5 sequences from soil. Lett Appl Microbiol 13: 21-24
12. Steffan RJ, Atlas RM (1988) DNA amplification to enhance detection of genetically engineered bacteria in environmental samples. Appl Environ Microbiol 54: 2185-2191
13. Porteous LA, Armstrong JL (1991) Recovery of bulk DNA from soil by a rapid, small-scale extraction method. Curr Microbiol 22: 345-348
14. Zhou J, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. Appl Environ Microbiol 62: 316-322
15. Boivin-Jahns V, Ruimy R, Bianchi A, Daumas S, Christen R (1996) Bacterial diversity in a deep-subsurface clay environment. Appl Environ Microbiol 62: 3405-3412
16. Erb RW, Wagner-Dobler I (1993) Detection of polychlorinated biphenyl degradation genes in polluted sediments by direct DNA extraction and polymerase chain reaction. Appl Environ Microbiol 59: 4065-4073
17. Picard C, Ponsonnet C, Paget E, Nesme X, Simonet P (1992) Detection and enumeration of bacteria in soil by direct DNA extraction and polymerase chain reaction. Appl Environ Microbiol 58: 2717-2722
18. Tsai YL, Olson BH (1991) Rapid method for direct extraction of DNA from soil and sediments. Appl Environ Microbiol 57: 1070-1074
19. Roose-Amsaleg CL, Garnier-Sillam E, Harry M (2001) Extraction and purification of microbial DNA from soil and sediment samples. Appl Soil Ecol 18
20. Gray JP, Herwig RP (1996) Phylogenetic analysis of the bacterial communities in marine sediments. Appl Environ Microbiol 62: 4049-4059
21. Leff LG, Dana JR, McArthur JV, Shimkets LJ (1995) Comparison of methods of DNA extraction from stream sediments. Appl Environ Microbiol 61: 1141-1143
22. Jacobsen CS, Rasmussen OF (1992) Development and Application of a New Method To Extract Bacterial DNA from Soil Based on Separation of Bacteria from Soil with Cation-Exchange Resin. Appl Environ Microbiol 58: 2458-2462